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'Telomeric RNA': End chromatin architecture's munificent merchandise

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ABSTRACT:
Linear chromosomes pose a general confront: how to protect the natural ends of chromosomes from breakdown, degradation, avoid recognition, processing as double-strand breaks and the rejoinder is "Telomere"! The conserved repetitive sequence of DNA also referred as "TEL Sequence" and enzyme "Telomerase" complete the cap of the chromosome "The Telomere". An added complicating factor is that the likelihood of a functional interaction between telomerase, telomeric proteins and repetitive DNA at telomeres is almost unquestionably regulated at the level of telomere chromatin which opens the field of an emerging research accompanied by much interest.
As a consequence, the present review focuses on ribonucleic acid transcribed from telomeric repetitive deoxyribonucleic acid and its future fascia. Untill now, telomeres were considered to be transcriptionally silent. However it has been found that telomeric DNA does transcribe into small oligonucleotides of RNA called telomeric RNA also called as Telomeric repeat containing RNA "TERRA". Due to its unique conformation it is involved in various biological functions concluding that majority of cellular functioning. Looking at the current facet, it is going to be boon in future and will not let a single stone unturned.

Key words: Telomere, Telomerase, TERRA, G-quadruplex, Telomeric RNA

INTRODUCTION

Telomeres consist of tandem TTAGGG repeats that cap and protect the chromosome ends. Telomeres shorten 50 – 200 bp with each cell division resulting from incomplete DNA replication of the lagging strand and other end-processing events, and this shortening can be overcome by the expression of telomerase enzyme [1]. The DNA component of telomeres is characterized in all vertebrates by tandem repeats of (TTAGGG/CCCTAA)n [2]. Telomeric DNA typically ends in a single-strand G-rich overhang of between 50 and 300 nucleotides at the 3' end, which has been proposed to fold back onto duplex telomeric DNA forming a "T-loop" structure [3].

COVERT ROLE OF TELOMERASE ENZYME:

Linear chromosomes pose a general challenge: how to protect the natural ends of chromosomes from breakdown, degradation, avoid recognition and processing as double-strand breaks. There are many different solutions to this problem, ranging from covalently closed hairpin ends in some viruses, bacteria, and phages [4] to specific transposable elements in certain insects [5]. However, in organisms as diverse as protozoan, fungi, mammals, and plants, telomeres consists of G-rich repetitive DNA maintained by a specialized reverse transcriptase enzyme called telomerase. Telomerase is a large ribonucleoprotein complex [6, 7] containing an RNA subunit [8, 9, 10, 11, 12, 13] and several protein components.

The RNA moiety is essential for the enzymatic function of telomerase. Besides serving as a template for reverse transcription in telomere DNA synthesis, the RNA subunit is also involved in the enzyme active site, probably with specific nucleotides interacting with structural components of the DNA primer substrate and protein subunits [14]. Removal or down-regulation of the RNA subunit leads to inhibition of telomerase, erosion of telomeres, compromise of growth capacity of highly proliferative embryonic stem cells [15], testicular cells, and hematopoietic cells [16] in the mouse, and death of both cultured HeLa cells [10] and malignant human glioma cells [17]. Although only a single gene for the telomerase RNA subunit has been identified, there appear to be at least three genes that have been cloned coding telomerase protein components in various organisms.

Telomerase is a specialized reverse transcriptase capable of extending the 3' end of chromosomes by adding TTAGGG repeats [18, 19]. Human telomerase RNA (hTR) is 445 nucleotides long with an 11 nucleotide putative template sequence (59-CUAACCCUAAC-39) coding for the telomere repeats of (TTAGGG)n [7]. The human core enzyme consists of a reverse transcriptase protein (TERT) of 1,132 amino acids encoded by the hTERT gene [20, 21, 22] located on chromosome 5p15.33.

The ribonucleoprotein dyskerin (encoded by the DKC1 gene on the X chromosome) is required for proper folding and stability of telomerase RNA [23] and was recently found to be part of the basic human telomerase enzyme complex [24]. Both the reverse transcriptase and telomerase RNA are expressed at very low levels, and haplo-insufficiency for either gene or mutations in DKC1 can give rise to various clinical

manifestations. Telomerase levels are regulated at multiple levels including transcription, alternative splicing, assembly, subcellular localization, and posttranslational modifications of various components and of the enzyme complex itself.

The relative importance of the various factors that have been proposed to affect the activity of telomerase at telomeres is difficult to discern, and the relative importance of such factors could vary between cell types. Another complicating factor is that the likelihood of a functional interaction between telomerase and repetitive DNA at telomeres is almost certainly also regulated at the level of telomere chromatin, an emerging research topic of much interest.

TELOMERIC PROTEIN: ROLE IN THE WORLD OF TELOMERE

A large number of proteins have been found to directly or indirectly associated with telomeric DNA. Some of these proteins, such as TRF1, TRF2, TIN2, TPP1, Rap1, and POT1 [25], can be found at telomeres at any time, although the dynamic exchange between telomere bound and unbound proteins can be high. Other important telomere proteins or protein complexes, such as the telomerase enzyme complex, associate with telomeric DNA only transiently. Much progress has been made in the last decade regarding the characterization of specific proteins at telomeres and their role in telomere function [25]. Many proteins that are known to (transiently) associate with telomeric DNA have roles outside telomeres, and the factors regulating their interactions and traffic are incompletely dephosphorylation, poly-ADP ribosylation, and deribosylation, acetylation, ubiquitination, sumoylation, etc., are crucial for the accumulation of specific proteins at telomeres during specific stages of the cell cycle.

Many “telomeric” proteins can be found at cytoplasmic and nontelomeric nuclear sites, and some proteins appear to localize at telomeres for yet unknown reasons. In general, the “cross-talk” between the many proteins involved in telomere function and various cellular signaling pathways is poorly understood. Challenges are differences in the recruitment of specific proteins to telomeric DNA between primary (diploid) cell types and the immortalized cell lines that are typically studied in the laboratory. Such differences complicate generalizations about the function of proteins that have been found to associate with telomeric DNA.

Human chromosome ends are typically capped with between 0.5 and 15 kilobase (kb) pairs of detectable telomere repeats depending on the type of tissue, the age of the donor, and the replicative history of the cells. Telomeres prevent the ends of linear chromosomes from appearing as DNA double strand (ds) breaks and protect chromosome ends from degradation and fusion. It has been proposed that telomeres can switch between an open state (in principle allowing elongation by telomerase) and a closed state (inaccessible to telomerase) with the likelihood of the open state inversely related to the length of the repeat tract [26].

TELOMERIC REPEAT CONTAINING RNA (TERRA)

TERRA is a heterogeneous non-coding RNA that consists of a combination of subtelomeric and telomeric sequences. To date, all individual telomeres tested in mammals can and do produce TERRA transcripts and TERRA is expressed in most tissues [27,28, 29]. TERRA is transcribed in a centromere to telomere direction, indicating that the transcription start site lies within the subtelomeric sequence. In the yeast, *Saccharomyces cerevisiae*, it has been determined that TERRA transcription occurs at both, telomeres containing or lacking the Y’ element. The Y’ element is a conserved repeat sequence, present at~ 50% of all telomeres in *S. cerevisiae*. The 5’ end of TERRA derived from telomeres containing the Y’ element is relatively homogenous, supporting the idea of a defined start site [30]. This suggests that the heterogeneity of TERRA stems from its 3’ end, and indicates either that transcription can terminate in multiple places within the telomeric tract, or that TERRA 3’ ends are differentially processed. Although RNAPII has a major function in promoting TERRA transcription, there may be another RNA polymerase (either RNAPI or RNAPIII) that could potentially have a function in telomeric transcription in mammals. The idea of another RNA polymerase being involved in generation of TERRA would be consistent with recent data in which several subunits of RNAPI and RNAPIII (RPABC1, RPAC1, RPA49, RPA2 and RPA1) were found enriched in formaldehyde-crosslinked and partially purified human telomere fractions [31].

Most products of RNAPII transcription, TERRA is polyadenylated at its 3’ end [28, 30, 29]. Approximately 7% of human TERRA is polyadenylated [28], whereas most or all yeast TERRA molecules carry a poly (A) tail [30]. It is unknown whether TERRA polyadenylation involves the 3’ end cleavage of a longer TERRA precursor or whether terminated transcripts are directly polyadenylated. A canonical 5’-AAUAAA-3’ cleavage and a polyadenylation signal is not present at mammalian TERRA 3’-ends, whereas the telomere-derived GU-rich TERRA 3’ ends in yeast bear some resemblance to the canonical U-rich 3’ end-processing signals in this organism.

In yeast, the canonical polyadenylation polymerase, Pap1, is responsible for poly(A) addition and it most

likely contributes to the stability of the RNA, because TERRA becomes completely destabilized in pap1-1 temperature- sensitive mutants and polyadenylated TERRA is no longer detectable [30]. How the 5' end of TERRA is modified has not yet been addressed. If it contains a 7-methylguanosine (m7G) cap structure as do mRNAs, it likely gets uncapped in *S. cerevisiae* at the time when it becomes a target for Rat1-dependent degradation, which recognizes 5'- uncapped mono-phosphate structures. Although the specific transcription factors that are responsible for the transcriptional regulation of TERRA have not yet been elucidated, there are indications that some of the telomere bound proteins that make up the protective cap of telomeres, along with the heterochomatic markers present at telomeres, are involved in controlling TERRA levels.

Shelterin is a complex composed of six proteins (TRF1, TRF2, Rap1, TIN2, TPP1 and POT1), which binds to and protects telomeres [32]. Interestingly, TRF1 can interact with RNAPII as shown through co-immunoprecipitation experiments [29]. It has been reported that when TRF1 was depleted using small interfering RNA (siRNA), overall levels of TERRA decreased twofold, which suggests that TRF1 supports TERRA transcription [29]. It is unlikely; however, that TRF1 acts as a conventional transcriptional activator. First, depletion of TRF1 does not result in a corresponding loss of RNAPII associated with telomeric DNA. Second, TRF1 is restricted to the telomeric tract and likely does not spread into the subtelomere, wherein transcription is presumed to initiate. Finally, the overproduction of TRF1 results in less TERRA production, rather than more, although it should be noted that telomeres shorten in length upon TRF1 overexpression and this may also influence TERRA transcription [33,29,34].

TERRA AT TELOMERES

TERRA is exclusively found in nuclear RNA fractions from human and mouse cells, and is likely restricted to the nucleus in yeast as well. The fact that TERRA associates with telomeres suggests that TERRA may be an integral part of the telomere and could potentially be important for structural integrity. However, because TERRA is detected by FISH in only a subset of telomeres, it seems more likely that TERRA is not an essential or a permanent constituent of the telomeric chromatin, but rather has a transient structural function during telomere assembly or it may have regulatory roles in a subset of telomeres that may correspond to a specific functional state. Although TERRA remains associated with telomeres, it has not been shown whether TERRA molecules can move from one telomere to another or whether the RNA remains associated with the telomere that it was transcribed from.

There are two potential means by which TERRA could be tethered to telomeres. One possibility would be through interactions of a telomeric protein with the RNA or, alternatively, through a direct interaction of TERRA with the telomeric DNA. Both modes of association are possible and are indeed not mutually exclusive. In yeast, mainly indirect evidence has hinted that DNA–RNA hybrid formation is occurring to some extent. It was shown that overexpression of RNaseH, which degrades the RNA moiety in a DNA–RNA hybrid, could specifically reduce TERRA levels in a genetic background in which TERRA accumulates [30]. Furthermore, it was shown *in vitro* that telomeric DNA and telomeric RNA together form an intermolecular heat-stable G-quadruplex structure [35]. To date, there is no evidence that telomeric proteins are involved in the tethering of TERRA to chromosome ends.

G-QUADRUPLEX

As early as 1962, it was proposed that four guanine monophosphate dianions can assemble coplanarly stabilized by two hydrogen bonds per base [36], later termed as G-tetrad or G-quartet. These tetramolecular arrangements then prefer to consecutively stack on top of each other due to strong unpolar attractions resulting in a four-stranded helical conformation known as the G-quadruplex motif. Similar multi-stranded arrangements were proposed for poly-guanylic acid sequences stabilized by Na^+ ions, shortly thereafter in 1963 [37]. Though initially studied for structural information, in the last two decades these higher order nucleic acid conformations have been proposed to be involved in various biological functions such as gene regulation [38,39], nucleosome positioning [40,41], recombination [42], and genomic maintenance [43]. However, studies proving their in vivo existence are limited [44, 45].

Duplex DNA is the thermodynamically favorable state compared to the G-quadruplex motif in the presence of a complementary strand [46, 47]. The single-stranded 3'-overhang of the telomeric repeat, which has been shown to adopt G-quadruplex structures *in vitro* [48], is the most studied G-quadruplex-forming sequence. However, it has been shown that 'capping' of the telomere end involves structures like the 't-Ioop' [3] as well as protein-ssDNA interactions [49] which are compromised by G-quadruplex formation. On the other hand, single stranded mRNA sequences harboring guanosine-rich stretches could also potentially form four-stranded structures in vivo. Proteins capable of inducing or stabilizing DNA and RNA G-quadruplexes have been described, which in turn may have functional relevance [50].

Since various genomic G-quadruplex forming regions are transcribed, it is interesting to compare stability parameters between DNA and RNA quadruplexes

especially. In general, slightly increased Ti/2 values were found for RNA quadruplexes compared to the corresponding DNA counterparts. The disparity is believed to arise from better stacking of G-tetrads and the 2'-OH group is able to form additional intramolecular hydrogen bonds in RNA quadruplexes [51]. Moreover, the stability could also result from higher-order scaffolding of RNA quadruplexes [52, 53, 54]. Though partially less stable than RNA quadruplexes, as early as 1994 [55] the telomeric DNA quadruplex was found to interfere with telomerase recognition and activity (telomeric repeat amplification protocol, TRAP assay) and since then the method has been extensively used to evaluate quadruplex formation and stability.

Understanding how G-quadruplex (G4) DNA structures that form in G-rich tracts of the genome affect chromosomal stability and processes such as copying the genetic information (DNA replication) or decoding the information (RNA transcription) has posed a significant challenge to researchers in the field. Although historically there has been some controversy over the existence of G4 DNA structures in vivo, emerging evidence suggests that they are indeed likely to form and have cellular consequences. In a recent study, Smith *et al* [56] investigated a role of G4 DNA in telomere capping, i.e., the adaptation of a nucleoprotein structure that prevents the chromosomal DNA ends from being recognized as DNA breaks and protects them from becoming degraded or fused.

TELOMERIC RNA

Human telomeres consist of tandem 5'-GGGTTA-3' repeats and have been shown to form G-quadruplex motifs under *in vitro* conditions [57, 58]. Telomere capping is a fairly complex process since a number of proteins have been shown to bind telomeric single-stranded or double-stranded DNA at the chromosome end. Moreover, the ability of telomeric DNA to form a variety of conformations including t-loops and G-quadruplexes adds to the complexity of how competing proteins and DNA structures influence the structural topology and metabolism of chromosome ends [59].

Interestingly, due to the absence of protein-coding genes and the heterochromatin nature of the telomeric and subtelomeric regions, it was believed that telomeres are transcriptionally inactive. In particular, an eGFP gene when inserted at the subtelomeric region was found to be silenced, confirming repressive chromatin architecture [60]. Nonetheless, in a recent northern blot analysis of whole-cell RNA using a telomere specific probe, a ~ 100-9,000 bp long telomeric repeat-containing RNA (termed TERRA) was found [27], indicating that telomeres are actively transcribed. Besides, RNA-FISH (fluorescence in situ hybridization) experiments revealed that TERRA is co-localized with the telomere region. Recently, the telomeric RNA repeat has also been shown to form an all-parallel RNA-quadruplex motif *in vitro* [61, 29, 62]. Although so far the non-coding TERRA has not been assigned any functional role, it is proposed to negatively regulate telomerase activity in a telomere-length-dependent manner [29], potentially by forming an RNA-DNA duplex [35].

The G-rich telomeric RNA contains repeats of three guanines, a motif that has been shown to form G-quadruplexes in single-stranded DNA [63,64, 57,65]. G-quadruplexes consist of multiple quartets of guanine bases hydrogen-bonded to one another and associated with either a monovalent or a divalent cation. If G-quadruplexes were to form in the G-rich telomeric RNA, this would result in a very different structure as contrasted to the C-rich telomeric RNA, which would not be predicted to form similar structures. G-quadruplexes may shield the G-rich telomeric transcripts from nucleases, as well as providing a conformation different from normal transcripts. Single stranded DNA-containing tracts of guanines can fold into a variety of quadruplex structures with the strands folding into parallel or antiparallel arrangements, depending on sequence and ionic environment [63, 66].

Studies of short G-rich telomeric DNA oligonucleotides provide a guide for understanding the structure of short G-rich telomeric RNA transcripts, less is known about G-quadruplex formation in RNA at lengths consistent with those found in vivo. Furthermore, RNA can be stabilized by base pairs that are not allowed in DNA, and runs of guanines in RNA may be sterically inhibited from forming antiparallel quadruplexes [67, 68]. Finally, the telomeric RNA transcripts in human cells range from ~100 nucleotides to nearly 9000 nucleotides, and such long RNA molecules could form complex structures not possible with short RNA molecules [27, 29].

CURRENT FACET AND FUTURE DIRECTIONS

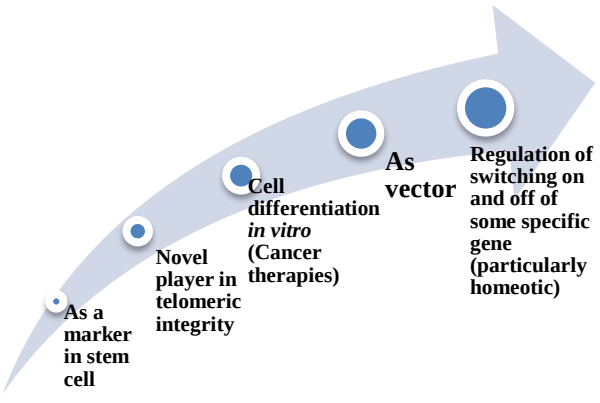

Figure 1: Current facet and future directions

AS A MARKER IN STEM CELL

Telomeric regions are known to be transcribed in several organisms. Although originally reported [69] to be transcribed from all chromosomes with enrichment near the inactive X of female cells, they showed that telomeric RNAs in fact are enriched on both sex chromosomes of the mouse in a developmentally specific manner. In female stem cells, both active Xs are marked by the RNAs. In male stem cells, both the X and the Y accumulate telomeric RNA. Distribution of telomeric RNAs changes during cell differentiation, after which they associate only with the heterochromatic sex chromosomes of each sex. FISH mapping suggests that accumulated telomeric RNAs localize at the distal telomeric end. Interestingly, telomeric expression changes in cancer and during cellular stress. Furthermore, RNA accumulation increases in Dicer-deficient stem cells, suggesting direct or indirect links to RNAi. They proposed that telomeric RNAs are tied to cell differentiation and may be used to mark pluripotency and disease.

NOVEL PLAYER IN TELOMERIC INTEGRITY

The identification of TERRA promoter regions and transcription start sites will increase the understanding of TERRA biogenesis. This information will help to understand whether TERRA RNA is required for the heterochromatinization of eukaryotic telomeres, similar to what has been observed for X-chromosome inactivation in females via Xist RNA (X inactive-specific transcript), another long non-coding RNA [70]. From the patho-physiological perspective, unravelling the role of TERRA at telomeres will enhance the insight into processes such as aging and cancer.

CELL DIFFERENTIATION IN VITRO (CANCER THERAPIES)

The link between telomere biology and oncogenesis was first proposed when telomerase expression was found to be a hallmark of human cancer: telomerase expression or reexpression and activity can be detected in 90% of tumor samples [71]. Telomere maintenance and telomerase reactivation is essential for the transformation of most human cancer cells. Telomere shortening to the threshold length, mutations of the telomere associated proteins, and/or telomerase RNA lead to telomeric dysfunction and therefore genetic instability. Telomerase up regulation in 85% of human cancer cells has become a hallmark of cancers, hence a promising target for anticancer therapy [72].

Telomeres are complexes at the termini of linear eukaryotic chromosomes that play a critical role in maintaining chromosomal integrity. Mammalian telomeres consist of hexanucleotide TTAGGG repeats (with an average length of 5–15 kb in humans) and associated protein components. This DNA-complex functions to “cap” the chromosome ends that protect against events such as end-end fusion and assure that telomere ends are not recognized as DNA breaks that trigger repair, cell cycle arrest, or apoptosis. In the absence of compensatory mechanisms, telomeric DNA shortens with each cell division, reflecting incomplete synthesis of telomeric termini during chromosomal replication. When telomeres reach a critically short length, cells enter a state that is termed replicative senescence in which they are incapable of further division and may be susceptible to increased apoptotic cell death [73]. This finite replicative capacity is a characteristic of most normal human somatic cells. In contrast, however, cancer cells, cells of the germ line, and some somatic cell populations escape or delay replicative senescence through expression of telomerase, a unique enzyme that is capable of synthesizing telomeric repeats [74].

AS VECTOR

Since telomere consists of repetitive sequence of nucleotides, the transcript of it has also conserved repetitive sequence. This RNA transcript may serve as a vector.

REGULATION OF SWITCHING ON AND OFF OF SOME SPECIFIC GENE (PARTICULARLY HOMEOTIC)

The genome is extensively transcribed into long intergenic noncoding RNAs (lincRNAs), many of which are implicated in gene silencing [75, 76]. Potential roles of lincRNAs in gene activation are much less understood [77, 78, 79]. Development and homeostasis require coordinate regulation of neighbouring genes through a process termed locus control [80]. Some locus control elements and enhancers transcribe lincRNAs [81, 82, 83, 84] hinting at possible roles in long-range control. In vertebrates, 39 Hox genes, encoding homeodomain transcription factors critical for positional identity, are clustered in four chromosomal loci; the Hox genes are expressed in nested anterior-posterior and proximal-distal patterns colinear with their genomic position from 3′ to 5′ of the cluster [85].

In addition, telomere repeat-containing RNAs (TERRAs) or telomeric RNAs (TelRNAs), which are RNAs that originate from telomeric DNA transcription, can associate with the telomeric chromatin, where they are proposed to function as negative regulators of telomere length based on their ability to act as potent inhibitors of telomerase *in vitro* [86, 27, 29]. Telomeric chromatin is dynamic. Differentiated somatic cells can be reverted to a more pluripotent state to become induced pluripotent stem (iPS) cells through a mechanism known as nuclear reprogramming [87]. The generation of iPS cells involves changes in the epigenetic status of telomeres

towards a more open chromatin conformation with a lower density of heterochromatic histone marks, which coincides with increased levels of TERRA, increased telomere recombination and continuous telomere elongation until reaching ES cell telomere length [88]. Although the regulation of telomere lengthening in a chromatin status-dependent manner has not been demonstrated, the observations described above strongly support this possibility.

REFERENCES:

[1] Shay J.W., W.E. Wright (2002) Telomerase: a target for cancer therapeutics. Cancer Cell, 2:257–265

[2] Moyzis R.K., J.M. Buckingham, L.S. Cram, M. Dani, L.L. Deaven, M.D. Jones, J. Meyne, R.L. Ratliff, J.R. Wu (1988) A highly conserved repetitive DNA sequence, (TTAGGG)n, present at the telomeres of human chromosomes. PNAS USA, 85:6622–6626

[3] Griffith J.D., L. Comeau, S. Rosenfield, R.M. Stansel, A. Bianchi, H. Moss, T. DeLange (1999) Mammalian telomeres end in a large duplex loop. Cell, 97:503–514

[4] Kobryn K., G. Chaconas (2001) The circle is broken: telomere resolution in linear replicons. Curr Opin Microbiol., 4:558–564

[5] Pardue M.L., S. Rashkova, E. Casacuberta, P.G. DeBaryshe, J.A. George, K.L. Traverse (2005) Two retrotransposons maintain telomeres in *Drosophila.* Chromosome Res., 13:443–453

[6] Greider C.W., E.H. Blackburn (1985) Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. Cell, 43:405–413

[7] Morin G.B. (1989) The human telomere terminal transferase enzyme is a ribonucleoprotein that synthesizes TTAGGG repeats. Cell, 59:521–9

[8] Greider C.W., E.H. Blackburn (1989) A telomeric sequence in the RNA of *Tetrahymena* telomerase required for telomere repeat synthesis. Nature (London), 337:331–337

[9] Shippen-Lentz D., E.H. Blackburn (1990) Functional evidence for an RNA template in telomerase. Science, 247:546–552

[10] Feng J., W.D. Funk, S.S. Wang, S.L. Weinrich, A.A. Avilion, C.P. Chiu, R.R. Adams, E. Chang, R.C. Allsopp, J. Yu, S. Le, M.D. West, C.B. Harley, W.H. Andrews, C.W. Greider, B. Villeponteau (1995) The RNA component of human telomerase. Science, 269:1236–1241

[11] Blasco M.A., M. Rizen, C.W. Greider, D. Hanahan (1996) Differential regulation of telomerase activity and telomerase RNA during multi-stage tumorigenesis. Nat Genet, 12:200–204

[12] Avilion A.A., M.A. Piatyszek, J. Gupta, J.W. Shay, S. Bacchetti, C.W. Greider (1996) Human telomerase RNA and telomerase activity in immortal cell lines and tumor tissues. Cancer Res., 56:645–650

[13] Tsao D.A., C.W. Wu, Y.S. Lin (1998) Molecular cloning of bovine telomerase RNA. Gene, 221:51–58

[14] Roy J., T.B. Fulton, E.H. Blackburn (1998) Specific telomerase RNA residues distant from the template are essential for telomerase function. Genes Deve., 12:3286–3300

[15] Niida H., T. Matsumoto, H. Satoh, M. Shiwa, Y. Tokutake, Y. Furuichi, Y. Shinkai (1998) Severe growth defect in mouse cells lacking the telomerase RNA component. Nat Genet, 19:203–206

[16] Lee H.W., M.A. Blasco, G.J. Gottlieb, J.W. Horner, C.W. Greider, R.A. DePinho (1998) Essential role of mouse telomerase in highly proliferative organs. Nature (London), 392:569–574

[17] Kondo S., Y. Tanaka, Y. Kondo, M. Hitomi, G.H. Barnett, Y. Ishizaka, J. Liu, T. Haqqi, A. Nishiyama, B. Villeponteau, J.K. Cowell, B.P. Barna (1998) Antisense telomerase treatment: induction of two distinct pathways, apoptosis and differentiation. FASEB J, 12:801–811

[18] Autexier C., N.F. Lue (2006) The structure and function of telomerase reverse transcriptase. Annu Rev Biochem, 75:493–517

[19] Collins K. (2006) The biogenesis and regulation of telomerase holoenzymes. Nat Rev Mol Cell Biol, 7:484–94

[20] Harrington L., T. McPhail, V. Mar, W. Zhou, R. Oulton, M.B. Bass, I. Arruda, M.O. Robinson (1997) A mammalian telomerase-associated protein. Science, 275:973–977

[21] Meyerson M., C.M. Counter, E.N. Eaton, L.W. Ellisen, P. Steiner, S.D. Caddle, L. Ziaugra, R.L. Beijersbergen, M.J. Davidoff, Q. Liu, S. Bacchetti, D.A. Haber, R.A. Weinberg (1997) hEST2, the putative human telomerase catalytic subunit gene, is up-regulated in tumor cells and during immortalization. Cell, 90:785–795

[22] Nakamura T.M., G.B. Morin, K.B. Chapman, S.L. Weinrich, W.H. Andrews, J. Lingner, C.B. Harley (1997) Telomerase catalytic subunit homologs from fission yeast and human. Science, 277:955–959

[23] Wong J.M., K. Collins (2006) Telomerase RNA level limits telomere maintenance in X-linked dyskeratosis congenital. Genes Dev, 20:2848– 2858

[24] Cohen S.B., M.E. Graham, G.O. Lovrecz, N. Bache, P.J. Robinson, R.R. Reddel (2007) Protein composition of catalytically active human telomerase from immortal cells. Science, 315:1850–1853

[25] DeLange T. (2005) Shelterin: the protein complex that shapes and safeguards human telomeres. Genes Dev, 19:2100–10

[26] Blackburn E.H. (2001) Switching and signaling at the telomere. Cell, 106:661–73

[27] Azzalin C.M., P. Reichenbach, L. Khoriauli, E. Giulotto, J. Lingner (2007) Telomeric repeat containing RNA and RNA surveillance factors at mammalian chromosome ends. Science, 318:798–801

[28] Azzalin C.M., J. Lingner (2008) Telomeres: the silence is broken. Cell Cycle, 7:1161–1165

[29] Schoeftner S., M.A. Blasco (2008) Developmentally regulated transcription of mammalian telomeres by DNA-dependent RNA polymerase II. Nat Cell Biol.,10: 228–236

[30] Luke B., A. Panza, S. Redon, N. Iglesias, Z. Li, J. Lingner (2008) The Rat1p 50 to 30 exonuclease degrades telomeric repeat-containing RNA and promotes telomere elongation in *Saccharomyces cerevisiae*. Mol Cell, 32:465–477

[31] Dejardin J., R.E. Kingston (2009) Purification of proteins associated with specific genomic loci. Cell, 136:175–186

[32] Palm W., T. DeLange (2008) How shelterin protects mammalian telomeres. Ann Rev Genet., 42:301–334

[33] Benetti R., S. Schoeftner, P. Munoz, M.A. Blasco (2008) Role of TRF2 in the assembly of telomeric chromatin. Cell Cycle, 7:3461–3468

[34] Munoz P., R. Blanco, G. DeCarcer, S. Schoeftner, R. Benetti, J.M. Flores, M. Malumbres, M.A. Blasco (2009) TRF1 controls telomere length and mitotic fidelity in epithelial homeostasis. Mol Cell Biol., 29:1608–1625

[35] Xu Y., T. Kimura, M. Komiyama (2008) Human telomere RNA and DNA form an intermolecular G-quadruplex. Nucleic Acids Symp Ser (Oxf), 52:169–170

[36] Gellert M., M.N. Lipsett, D.R. Davies (1962) Helix Formation By Guanylic Acid. PNAS USA, 48:2013-2018

[37] Fresco J.R., J. Massoulie (1963) Polynucleotides. V. Helix-Coil Transition of Polyriboguanylic Acid. 1. Am. Chem. Soc., 85:1352-1353

[38] Du Z., Y. Zhao, N. Li (2008) Genome-wide analysis reveals regulatory role of G4 DNA in gene transcription. Gene Res., 18:233-241

[39] Rawal P., V.B.R. Kurnrnarasetti, J. Ravindran, N. Kurnar, K. Halder, R. Sharrna, M. Mukerji, S.K. Das, S. Chowdhury (2006) Genome-wide prediction of G4 DNA as regulatory motifs: Role in *Escherichia coli* global regulation. Genome Res., 16:644- 655

[40] Halder K., R. Halder, S. Chowdhury (2009) Genome-wide analysis predicts DNA structural motifs as nucleosome exclusion signals. Mol BioSys., 5:1703-1712

[41] Wong H.M., J.L. Huppert (2009) Stable G-quadruplexes are found outside nucleosome-bound regions. Mol BioSys., 5:1713-1719

[42] Mani P., V.K. Yadav, S.K. Das, S. Chowdhury (2009) Genome-wide analyses of recombination prone regions predict role of DNA structural motif in recombination. PLoS One, 4:e4399

[43] Maizels N. (2006) Dynamic roles for G4 DNA in the biology of eukaryotic cells. Nat. Struct. Mol Biol., 13:1055-9

[44] Siddiqui-Jain A., C.L. Grand, D.J. Bearss, L.H. Hurley (2002) Direct evidence for a G-quadruplex in a promoter region and its targeting with a small molecule to repress c-*MYC* transcription. PNAS, 99:11593-11598

[45] Paeschke K., T. Simonsson, J. Postberg, D. Rhodes, H. Lipps (2005) Telomere end-binding proteins control the formation of G-quadruplex DNA structures *in vivo*. Nat. Struct. Mol Biol., 12:847-854

[46] Kurnar N., S. Maiti (2008) A thermodynamic overview of naturally occurring intramolecular DNA quadruplexes. Nucleic Acids Res., 36:5610-5622

[47] Risitano A., K.R. Fox (2003) Stability of Intramolecular DNA Quadruplexes: Comparison with DNA Duplexes. Biochem., 42:6507·-6513

[48] Sen D., W. Gilbert (1988) Formation of parallel four-stranded complexes by guanine-rich motifs in DNA and its implications for meiosis. Nature, 334:364-366

[49] Horvath M.P., V.L. Schweiker, J.M. Bevilacqua, J.A. Rllggles, S.C. Schultz (1998) Cell, 95:963-974

[50] Fry M. (2007) Tetraplex DNA and its interacting proteins. Front. Biosci., 12:4336-51

[51] Pagano B., C.A. Mattia, L. Cavallo, S. Uesugi, C. Giancola, F. Fraternali (2008 Stability and Cations Coordination of DNA and RNA 14-Mer G-Quadruplexes: A Multiscale Computational Approach. 1. Phys. Chem. B, 112:12115-12123

[52] Pan B., K. Shi, M. Sundaralingam (2006) Crystal Structure of an RNA Quadruplex Containing Inosine Tetrad: Implications for the Roles of NH_2 Group in Purine Tetrads. J Mol. Biol., 363:451-459

[53] Pan B., Y. Xiong, K. Shi, M. Sundaralingam (2003) Crystal Structure of a Bulged RNA Tetraplex at 1.1 Å Resolution: Implications for a Novel Binding Site in RNA Tetraplex. Structure, 11:1423-1430

[54] Liu H., A. Matsugami, M. Katahira, S. Uesugi (2002) A Dimeric RNA Quadruplex Architecture Comprised of Two G:G(:A):G:G(:A) Hexads, G:G:G:G Tetrads and UUUU Loops. J Mol Biol., 322:955-970

[55] Kim N.W., M.A. Piatyszek, K.R. Prowse, C.B. Harley, M.D. West, P.L. Ho, G.M. Coviello, W.E. Wright, S.L. Weinrich, J.W. Shay (1994) Science, 266:2011-2015

[56] Smith J.S., Q. Chen, L.A. Yatsunyk, J.M. Nicoludis, M.S. Garcia, R. Kranaster, S. Balasubramanian, D. Monchaud, M.P. Teulade-Fichou, L. Abramowitz, D.C. Schultz, F.B. Johnson (2011) Rudimentary Gquadruplex- based telomere capping in *Saccharomyces cerevisiae*. Nat. Struct. MolBiol., 2011:478-485

[57] Parkinson G., M. Lee, S. Neidle (2002) Crystal structure of parallel quadruplexes from human telomeric DNA. Nature, 417:876-880

[58] Xu Y., K. Kaminaga, M. Komiyama (2008) G-Quadruplex Formation by Human Telomeric Repeats-Containing RNA in Na^+ Solution. 1. Am. Chem. Soc.,130: 11179- 11184

[59] Paeschke K., K.R. McDonald, V.A. Zakian (2010) Telomeres: structures in need of unwinding. FEBS Lett., 584:3760-3772

[60] Koering C., A. Pollice, M. Zibella, S. Bauwens, A. Puisieux, M. Brunori, C. Brun, L. Martins, L. Sabatier, J. Pulitzer, E. Gilson (2002) Human telomeric position effect is determined by chromosomal context and telomeric chromatin integrity. EMBO Rep., 3:1055-1061

[61] Martadinata H., A.T. Phan (2009) Structure of Propeller-Type Parallel-Stranded RNA G-Quadruplexes, Formed by Human Telomeric RNA Sequences in K^+ Solution. 1. Am. Chem. Soc., 131:2570-2578

[62] Randall A., J.D. Griffith (2009) Structure of Long Telomeric RNA Transcripts: The G-Rich Rna Forms A Compact Repeating Structure Containing G-Quartets. J Biol Chem., 284:13980-13986

[63] Yu H.Q., D. Miyoshi, N. Sugimoto (2006) Characterization of Structure and Stability of Long Telomeric DNA G-Quadruplexes. 1. Am. Chem. Soc.**,** 128:15461–15468

[64] Wang Y., D.J. Patel (1993) Solution structure of the human telomeric repeat $d[AG_3(T_2AG_3)_3]$ G-tetraplex. Structure (Lond.), 1:263–282

[65] Phan A.T., V. Kuryavyi, K.N. Luu, D.J. Patel (2007) Structure of two intramolecular G-quadruplexes formed by natural human telomere sequences in K^+ solution. Nucleic Acids Res., 35:6517–6525

[66] Keniry M. (2000) Quadruplex structures in nucleic acids. Biopoly., 56:123–146

[67] Westhof E., V. Fritsch (2000) RNA folding: beyond Watson–Crick pairs. Structure (Lond.), 8:R55–R65

[68] Tang C.F., R.H. Shafer (2006) Engineering the Quadruplex Fold: Nucleoside Conformation Determines Both Folding Topology and Molecularity in Guanine Quadruplexes. 1. Am. Chem. Soc., 128:5966–5973

[69] Zhang L., Y. Ogawa, J. Ahn, S. Namekawa, S. Silva, J.T. Lee (2009) Telomeric RNAs mark sex chromosomes in stem cells. Genetics, 182:685–698

[70] Ng K., D. Pullirsch, M. Leeb, A. Wutz (2007) Xist and the order of silencing. EMBO Rep., 8:34-39

[71] Shay J.W., I.B. Roninson (2004) Hallmarks of senescence in carcinogenesis and cancer therapy. Oncogene, 23:2919–2933

[72] Tian X., X. Chen, X. Liu (2010) Telomere and telomerase as targets for cancer therapy. App Biochem. Biotechnol., 160:1460-1472

[73] Richard H. (2001) Molecular targeting of cancer: Telomeres as targets. PNAS, 98:7649–51

[74] McEachern M.J., A. Krauskopf, E.H. Blackburn (2000) Telomeres and their control. Ann. Rev.genet., 34:331–358

[75] Mercer T.R., M.E. Dinger, J.S. Mattick (2009) Long non-coding RNAs: insights into functions. Nat. Rev Genet., 10:155–159

[76] Ponting C.P., P.L. Oliver, W. Reik (2009) Evolution and functions of long noncoding RNAs. Cell, 136:629–641

[77] Sanchez-Elsner T., D. Gou, E. Kremmer, F. Sauer (2006) Noncoding RNAs of trithorax response elements recruit Drosophila Ash1 to Ultrabithorax. Science, 311:1118–1123

[78] Petruk S., Y. Sedkov, K.M. Riley, J. Hodgson, F. Schweisguth, S. Hirose, J.B. Jaynes, H.W. Brock, A. Mazo (2006) Transcription of bxd noncoding RNAs promoted by trithorax represses Ubx in cis by transcriptional interference. Cell, 127:1209–1221

[79] Dinger M.E., P.P. Amaral, T.R. Mercer, K.C. Pang, S.J. Bruce, B.B. Gardiner, M.E. Askarian-Amiri, K. Ru, G. Soldà, C. Simons, S.M. Sunkin, M.L. Crowe, S.M. Grimmond, A.C. Perkins, J.S. Mattick (2008) Long noncoding RNAs in mouse embryonic stem cell pluripotency and differentiation. Gene Res., 18:1433–1445

[80] Dean A. (2006) On a chromosome far, far away: LCRs and gene expression. Trends in Genetics, 22:38–45

[81] Ashe H.L., J. Monks, M. Wijgerde, P. Fraser, N.J. Proudfoot (1997) Intergenic transcription and transinduction of the human b-globin locus. Genes Dev, 11:2494–2509

[82] DeSanta .F, I. Barozzi, F. Mietton, S. Ghisletti, S. Polletti, B.K. Tusi, H. Muller, J. Ragoussis, C.L. Wei, G. Natoli (2010) A large fraction of extragenic RNA Pol II transcription sites overlap enhancers. PLoSBiol, 8:e1000384

[83] Kim T.K., M. Hemberg, J.M. Gray, A.M. Costa, D.M. Bear, J. Wu, D.A. Harmin, M. Laptewicz, K. Barbara-Haley, S. Kuersten, E. Markenscoff-Papadimitriou, D. Kuhl, H. Bito, P.F. Worley, G. Kreiman, M.E. Greenberg (2010) Widespread transcription at neuronal activity-regulated enhancers. Nature, 465:182–187

[84] Ørom U.A., T. Derrien, M. Beringer, K. Gumireddy, A. Gardini, G. Bussotti, F. Lai, M. Zytnicki, C. Notredame, Q. Huang, R. Guigo, R. Shiekhattar (2010) Long noncoding RNAs with enhancer-like function in human cells. Cell, 143:46–58

[85] Chang H.Y. (2009) Anatomic demarcation of cells: genes to patterns. Science, 326:1206–1207

[86] Schoeftner S., M.A. Blasco (2009) 'higher order' of telomere regulation: telomere heterochromatin and telomeric RNAs. EMBO J., 28:2323–2336

[87] Takahashi K., S. Yamanaka (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 126: 663–676

[88] Marion R.M., K. Strati, H. Li, A. Tejera, S. Schoeftner, S. Ortega, M. Serrano, M.A. Blasco (2009) Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells. Cell Stem Cell, 4:141–154